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FORM PTO-1390 (Modified) (REV 11-2000) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **ABLE-0020**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/857097

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/04027	**1 December 1999**	**1 December 1998**

TITLE OF INVENTION: **Allo and Auto-Reactive T-Cell Epitopes**

APPLICANT(S) FOR DO/EO/US: **URBANIAK, Stanislaw Joseph et al.**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)) – unexecuted
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance wit
20. ☐ A second copy of the published international application under 35 U
21. ☐ A second copy of the English language translation of the internatio
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

1) Copy of Written Opinion and Response thereto;
2) Return Post Card

"Express Mail" Label No. **EL792267783US**
Date of Deposit **May 31,2001**

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

By Deborah Ehret
Typed Name: Deborah Ehret

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/857097	PCT/GB99/04027	ABLE-0020

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1000.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$860.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	23 - 20 =	3	x $18.00	**$54.00**	
Independent claims	10 - 3 =	7	x $80.00	**$560.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,474.00**	
☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$737.00**	
SUBTOTAL =				**$737.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE =				**$737.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$737.00**	
				Amount to be: refunded	$
				charged	$

Applicant, Aberdeen University is a University of higher education and therefore entitled to small entity status. Applicant, The Common Services Agency for the Scottish Health Service is a non-profit organization and therefore entitled to small entity status.

a. ☒ A check in the amount of **$737.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-1619** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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ALLO AND AUTO-REACTIVE T-CELL EPITOPES

The present invention relates to the mapping of allo-reactive T-cell epitopes on the rhesus(RhD and RhCc/Ee) proteins and to the use of such epitopes to modulate the corresponding immune responses to these antigens.

Human blood contains a genetically complex rhesus (Rh) blood group system. For example, humans are either RhD positive or negative and this can lead to problems during transfusions or pregnancy when RhD negative individuals are exposed to RhD positive blood and become immunised to produce anti-D.

The most important allele in the RhD blood group system is the D antigen. The RhD antigen is carried by the RhD protein which is a transmembrane protein consisting of 417 amino acids with 12 putative transmembrane domains and 6 extracellular loops. A series of peptides have been constructed in the present invention based on the RhD protein each being 15 amino acids (AA) long, and tested *in vitro* against T-lymphocytes from normal individuals, donors who have been alloimmunised to produce anti-D, and patients with warm type autoimmune haemolytic anaemia.

The full amino acid sequence of the RhCE polypeptide and the differences in sequence for c, e and D polypeptides is shown in Figure 1 hereinafter (Reference: The Blood Group Antigen Facts Book, p94, Editors; M E Reid & C Lomas-Francis, Academic Press London).

The complexity of the blood system can cause problems during pregnancy when a woman who is RhD negative is carrying a RhD positive foetus, as the woman is at risk of being immunized

by the RhD positive blood cells of her own baby. This immunisation can take place during situations when the mother's and baby's blood can become mixed, for example during amniocentesis, antepartum haemorrhage but mainly at parturition.

Once the mother's immune system has been exposed to RhD positive blood cells, she will produce anti-D antibodies which can cross the placenta and cause Rh haemolytic disease in any subsequent RhD positive pregnancies. Such haemolytic disease can be fatal for the neonate.

Currently, purified anti-D immunoglobulin is injected whenever a mother is exposed to fetal RhD positive red blood cells which may occur during e.g., amniocentesis, antepartum haemorrhage but mainly at parturition. About 17% of Caucasian women are RhD negative so that most industrialized countries have RhD prevention programmes wherein all RhD negative women receive prophylaxis with anti-D immunoglobulin at delivery or in association with the other high risk events alluded to above. Further in many countries, routine antepartum prophylaxis to minimize the incidence of Rh haemolytic disease is practised.

There are a number of problems with this approach. In the first place efficacy is never entirely complete since events can be missed or undeclared or a foetal haemorrhage can be larger than the anti-D can neutralize. Secondly, current anti-D immunoglobulin comes from deliberately immunised donors, which puts volunteers, often male (paid or not) at some small risk. In addition it takes at least 12 months to accredit the donors during which time their blood products are not available. For these reasons there is a worldwide

shortage of anti-D immunoglobulin. Finally, there are also concerns about the safety of recipients who may be exposed to transfusion transmitted infections such as by inadvertent infection with agents, for example variant Creutzfeld-Jacob Disease (vCJD) for which there is no satisfactory test.

Other groups that can be at risk from alloimmunisation are those who are regular recipients of bloods products, for example those suffering from haemological malignant disease, sickle cell disease or thalassaemia.

Certain RhD peptides have been found to specifically stimulate the helper T-cells of alloimmunised individuals. Conversely, certain RhD peptides have been found to stimulate the production of immunosuppressive cytokines by helper T-cells. There is furthermore some correlation between the HLA-DR type of allo- and auto-immunised donors and the peptides which stimulate helper T-cell responses.

An object of the present invention is to provide an effective treatment for subjects that have become alloimmunised or have an autoimmune disease against red blood cells.

A further objective of the invention is to provide an effective prophylactic to prevent alloimmunisation.

In a first embodiment of the invention there is provided a pharmaceutical composition for the prevention of alloimmunisation of a subject, said composition comprising an immunologically effective epitope of a rhesus protein or an immunologically active analogue or derivative thereof.

We have mapped helper T-cell epitopes on the RhD protein. The characterization of a helper epitope that is targeted in most alloimmunised donors and the identification of correlations between HLA-DR type and particular dominant epitopes opens the way for the evaluation of peptide immunotherapy as a novel way to regulate the immune response to RhD and to prevent Rh haemolytic disease and anti-D related transfusion problems.

Currently, anti-D which is given to pregnant women during significant events in pregnancy may be considered as a passive form of immunotherapy because it has the effect of blocking the effects of immune events on a temporary basis.

The replacement of passive with active peptide immunotherapy in RhD negative women is an attractive option since safe synthetic tolerogens can be developed and given before pregnancy thus avoiding foetal exposure. Suppression throughout pregnancy would mean that only one injection was necessary, considerably simplifying management of RhD negative women and also it may be possible for the first time to reverse rather than prevent alloimmunisation by administration of tolerogenic peptides to individuals who already have produced anti-D with the objective of "switching-off" the immune response to RhD.

Tolerogenic peptides to other Rh antigens, as determined by the current invention, would be of equivalent value in preventing, or modifying the production of alloantibodies by the respective antigens, including (but not exclusively) RhC, Rhc, RhE and Rhe; and Rh50 (peptides are shown in Table 4) in autoimmune haemolytic anaemia.

Accordingly the categories of individual in whom prior immunization would be considered are as follows:-

(1) All women during their child bearing years; and

(2) regular recipients of blood products; who might be exposed to blood transfusion for example haemological malignant disease, sickle cell disease and thalassaemia.

Such a pharmaceutical composition can be given to expectant mothers with RhD negative blood and a RhD positive child in this respect, the composition would result in the mother not producing an immune response at any occasion when the foetuses blood comes in contact with her own immune system. In this connection, there is a reduced likelihood that any subsequent baby which is RhD positive would suffer from haemolytic disease.

The use of synthetic peptides in accordance with the present invention removes concerns about viral infection being transmitted either by anti-D immunoglobulin used for passive immunotherapy or by red blood cells given to volunteer recipients. The time consuming and expensive procedures required to validate accredited donors and donations are also important considerations.

In addition, by use of these compositions, volunteers who are often RhD negative men, can avoid the usual injection of red blood cells when they are deliberately immunised for the production of anti-D immunoglobulin.

In a second embodiment of the invention there is provided a pharmaceutical composition for the immunosuppression of a response elicited by alloimmunisation of a subject or an autoimmune haemolytic disease, said composition comprising an

immunologically effective epitope of a rhesus protein or an immunologically active analogue or derivative thereof.

If the immune system of an RhD negative mother has already been in contact with the blood from a RhD positive baby, such a composition can used during subsequent pregnancies with a RhD positive baby to reduce the likelihood of the baby suffering from RhD haemolytic disease.

In addition, such a composition can be given to patients who have accidentally been given an RhD positive blood transfusion when they are RhD negative. In this connection, the availability of such a composition reduces the need for very large doses of anti-D immunoglobulin for prophylaxis and the likelihood of becoming alloimmunised thereafter.

Preferably autoimmune disease is idiopathic or secondary autoimmune haemolytic anaemia mediated by 'warm-type' autoantibodies. The trigger for this autoimmune disease is unknown and therefore it may occur at anytime and results in the body producing autoantibodies of broad Rh group specificity which attack the bodies own red blood cells.

Conveniently the rhesus protein is either RhD, RhC, Rhc, RhE or Rhe protein.

These determine the main Rh-specific antigens found on the surface of a red blood cell.

In a preferred embodiment an epitope selected from at least one of numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth.

The aforementioned are the most common epitopes recognised by T-cells of alloimmunised subjects and those suffering from autoimmune haemolytic anaemia. In autoimmune haemolytic anaemia, the preferred epitopes are 2, 5, 14, 29, 31 and 38. Therefore induced tolerance to such epitopes would stop an immune response being mounted if they appear in the blood of the subject.

Preferably the epitope is either epitope 12A or 29 since epitope 12A is the most common epitope recognised by alloreactive T-cells, epitope 29 is most commonly recognised in autoimmune haemolytic anaemia.

Conveniently any of the epitopes or immunoreactive derivatives can be synthesised.

If the epitope sequences are artificially synthesised microbial contamination is negligible.

In a third embodiment of the invention there is provided a pharmaceutical composition for the induction of alloimmunisation of a subject, said composition comprising an immunologically effective epitope of a rhesus protein or an immunologically active analogue or derivative thereof disposed in a pharmacologically acceptable vehicle.

Preferably the rhesus protein is either RhD, RhC, Rhc, RhE or Rhe protein, conveniently an epitope selected from at least one of numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth.

Preferably the vehicle is selected such that the composition is in an injectable, oral, rectal, topical or spray-uptake form.

It is known that mammals may be tolerised to certain stimuli by taking in specific peptide fragments, for example from the nasal mucosa or via the gut. We propose that a good way of abolishing the immune response to RhD in recipient females prior to, during, or after pregnancy is to administer rhesus peptides via the mucosa such as the nasal, buccal, or anal mucosa or transdermally. The peptide fragments in accordance with the present invention will enter via mucosal tissues and effectively tolerise the subject without causing a full blown immune response which may well be the case should the peptide fragments of the present invention reach circulating blood system at the first instance.

In an injectable form the epitopes can be used to deliberately immunise the subject with an epitope which can for example produce IL-10 or TGF-β which have immunosuppressive effects.

The outcome of this approach is to develop a "vaccine" using Rh epitopes which will suppress the immune response to Rh proteins.

In a fourth embodiment of the invention there is provided a tolerising peptide fragment disposed in a pharmacologically effective vehicle, said vehicle being adapted for injection, oral, rectal via a suppository, topical or spray-uptake administration to the subject wherein the tolerising peptide fragment is selected from an epitope of either a RhD, RhC, Rhc, RhE or Rhe protein. Preferably the epitope is selected

from at least one of epitope numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth.

Thus the pharmaceutically acceptable vehicle may be adapted for transdermal or transmucosal administration or wherein said vehicle may be a formulation with an enteric coating for oral administration.

In a fifth embodiment of the present invention there is provided a method of tolerizing a subject which comprises administering to said subject a tolerising peptide fragment.

In a sixth embodiment of the present invention there is provided an epitope from a RhD, RhC, Rhc, RhE or Rhe protein selected from at least one of epitope numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39.

In a seventh embodiment of the present invention there is provided the use in the manufacture of a medicament for the tolerisation of a patient who may become alloimmunised comprising an epitope selected from a RhD, RhC, Rhc, RhE or Rhe protein or selected from at least one of epitope numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 disposed in a pharmaceutically acceptable vehicle therefor.

In an eighth embodiment of the invention there is provided the use in the manufacture of a medicament for the immunosuppression of an alloimmunised patient or a patient with warm-type autoimmune haemolytic anaemia comprising an epitope selected from a RhD, RhC, Rhc, RhE or Rhe protein or selected from at least one of epitope numbers 2, 5, 6, 6A,

10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 disposed in a pharmaceutically acceptable vehicle therefor.

In a ninth embodiment of the invention there is provided a method for determining the effect of an epitope from a rhesus protein on a human lymphocyte, *in vitro*, comprising the steps of:-

a) stimulating the lymphocyte with one or more epitope of a rhesus protein;

b) between 4 and 7 days later resuspending the cultures and transferring aliquots into plates prepared in the following manner;

c) washing the plate at least once with Hanks Buffered Salt Solution (HBSS);

d) coating each well in the plate with monoclonal anti-cytokine capture antibody;

e) blocking any non-specific binding using an appropriate solution;

f) incubating the plates with the lymphocyte culture for 12-36 hours at 30-40°C in an atmosphere of substantially 5% CO_2 and substantially 95% air;

g) washing the plates at least once with Tween/PBS;

h) introducing an appropriate biotinylated monoclonal detection antibody to each well and incubating for 30-60 min at room temperature;

I) washing the plates at least once with Tween/PBS;

j) introducing ExtrAvidin-alkaline phosphatase conjugate and incubating for 15-45 mins;

k) washing the plates again at least once with Tween/PBS;

l) developing the plates with p-nitrophenyl phosphate in 0.05M carbonate alkaline buffer pH9.6 added to each well; and

m) reading the absorbance at 405nm.

Traditionally, among other techniques, researchers have used a captive assay called ELISPOT to determine the amount of cytokines produced by a cell. This assay produces a colour spot for each cytokine producing cell. A crude calculation based on the number of coloured spots is then used to estimate the amount of cytokines produced. The use of p-nitrophenyl phosphate in the present assay allows the amount of cytokine captured by the antibody in the wall to be established on the basis of the colour change produced which can be measured by the more accurate method of spectrophotometry.

Accordingly, this method is very sensitive and therefore can identify that a particular RhD protein is capable of stimulating human T-cells to produce potentially immunosuppressive cytokines rather than to proliferate. This is important for the determination of the method of delivery of an epitope. An epitope which leads to T-cell proliferation may be given as a tolerogen through the nasal or mucosal route whereas an epitope which leads to immunosuppresive cytokines may be injected.

In a tenth embodiment of the present invention there is provided a method for the determination of the propensity of a RhD negative subject to produce anti-D antibodies after exposure to RhD positive blood comprising ascertaining the tissue type of the subject and determining if they are HLA-DRB1*15.

If the subject has a tissue type of HLA-DRB1*15 they are more likely to raise anti-D antibodies therefore they should be given treatment before being put at risk of exposure to RhD positive red blood cells.

The invention will now be described, by way of illustration only, with reference to the following examples and the accompanying figures.

Figure 1 shows the full amino acid sequence of RhCE polypeptide; differences in the sequence for Rhc, Rhe and RhD polypeptides are also shown (Reference: The Blood Group Antigen Facts Book P94, Editor; M E Reid & C Lomas-Francis, Academic Press London).

Figure 2 shows the distribution of stimulatory RhD peptides in donors alloimmunised with RhD antigen from peptides 1 to 42 and 6A to 40A as per Tables 1, 2 and 3; x - RhD peptide added to culture; y - percentage of subjects responding to specific RhD peptides.

Figure 3A shows the distribution of stimulating RhD peptides in autoimmune haemolytic anaemia patients; x - RhD peptide stimulus; y - percentage of subjects responding to specific RhD peptides.

Figure 3B shows the distribution of stimulating RhD peptides in normal controls; x - RhD peptide stimulus; y - percentage of subjects responding to specific RhD peptides.

Figure 4 shows the correlation between Rh epitopes recognised in donors sharing a tissue type. X and Y axes represent the stimulation indices for donors 1 and 2 respectively. Each square represents the response to a peptide. Correlation co-efficient (R) = 0.774, p value 9.57E-015

Figure 5A shows the response pattern to the induction of

TGF-β production of T-cells after incubation with Rh peptides; x - RhD peptide stimulus; y - TGF-β1 secretion (pg/ml). Value D = none.

Figure 5B shows the response pattern to the induction of IL-10 production of T-cells after incubation with Rh peptides; x - RhD peptide stimulus; y - IL-10 secretion (ng/ml). Value D = none.

Figure 5C shows the response pattern to the induction of IFN-γ production of T-cells after incubation with Rh peptides; x - RhD peptide stimulus; y - IFN-γ secretion (ng/ml). Value D = none.

Figure 5D shows the amount of incorporation of ^{3}H-Thymidine into T-cells after incubation with Rh peptides; x - RhD peptide stimulus; y - ^{3}H-Thymidine incorporation (mean CPMx10^{-3} ±SD) SI=3. Value D = none.

Figure 6 shows the inhibition of T-cells that respond to RhD protein by peptides that generate an immunosuppressive cytokine response; x - RhD peptide stimulus; y - ^{3}H-Thymidine incorporation (mean CPMx10^{-3} ±SD). A - none; B - control (-); C - RhD; D - RhD & 16; E - RhD & 22; F - RhD & 24; G - none; H - PPD; I - PPD & 16; J - PPD & 22; K - PPD & 24.

EXAMPLE 1

Two complete panels of 68 15-mer peptides, with 5 or 10 amino acid overlaps, were synthesized (Multiple Peptide Service, Cambridge Research Biochemicals, Cheshire, UK and Dept. Of Biochemistry, University of Bristol, UK), corresponding to the sequences of the 30kD Rh proteins associated with

expression of the RhD or RhCc/Ee blood group antigens respectively. The amino acid sequences for each of these proteins were deduced independently from cDNA analyses by 2 laboratories. Since the two polypeptide sequences show 92% homology, 16 of the synthetic peptides were shared between the panels (numbering from the amino terminus, peptides 1-5, 8, 9, 14, 21, 28, 29, 37-39, 41 and 42). In order to ensure purity, each panel was synthesized by fluorenylmethoxycarbonyl chemistry on resin using a base-labile linker, rather than by conventional pin technology, and randomly selected peptides were screened for purity by HPLC and amino acid analysis. The peptides were used to stimulate cultures at 20μg/ml, although it should be noted that the responses of the cultures had previously been shown to be similar in magnitude and kinetics at peptide concentration between 5-20μg/ml.

The control antigens *Mycohacterium tuberculosis* purified protein derivative (PPD) (Statens Seruminstut, Denmark) and keyhole limpet hemocyanin (KLH) (Calbiochem-Behring, La Jolla, Ca., USA) were dialysed extensively against phosphate buffered saline pH 7.4 (PBS) and filter sterilized before addition to cultures at 50μg/ml, PPD, but not KLH, readily provokes recall T-cell responses *in vitro*, since most UK citizens have been immunised with BCG. Concanavalin A (Con A) was obtained from Sigma, Poole, Dorset, UK, and used to stimulate cultures at 10μg/ml.

Antibodies

FITC- or phycoerythrin-conjugated mAbs against human CD3, CD19, CD45 or CD14 were obtained from Dako UK Ltd. Blocking mAbs specific for HLA-DP, -DQ, or -DR supplied by Becton

Dickinson (Oxford, UK) were dialysed thoroughly against PBS before addition to cultures at the previously determined optimum concentration of 2.5μg/ml.

Isolation of Peripheral Blood Mononuclear Cells and T-cells

Peripheral blood mononuclear cells (PBMC) from donors or patients were separated from fresh blood samples using Ficoll-Hypaque. The donors and patients had become alloimmunised with RhD positive blood either through pregnancy, a blood transfusion or through immunization with the relevant blood.

The viability of PBMC was greater than 90% in all experiments, as judged by trypan blue exclusion. T-cells were isolated from PBMC by passage through glass bead affinity columns coated with human IgG/sheep anti-human IgG immune complexes. Flow cytometry (Becton Dickinson FACScan) demonstrated that typical preparations contained more than 95% T-cells.

Cell Proliferation Assays

PBMC were cultured in 100μl volumes in microtitre plates at a concentration of 1.25×10^6 cells/ml in an Alpha Modification of Eagle's Medium (ICN Flow, Bucks UK) supplemented with 5% autologous serum, 4mM L-Glutamine (Gibco, Paisley, UK), 100U/ml sodium benzylpenicillin G (Sigma), 100μg/ml streptomycin sulphate (Sigma), 5×10^{-5}M 2-mercaptoethanol (Sigma) and 20mM HEPES pH7,2 (Sigma). All plates were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air. The cell proliferation in cultures was estimated from the incorporation of ^{3}H-Thymidine in triplicate

wells 5 days after stimulation with antigen as described previously. Proliferation results are presented either as the mean CPM +/- SD of the triplicate samples, or as a stimulation index (SI), expressing the ratio of mean CPM in stimulated versus unstimulated control cultures. An SI>3 with CPM>1000 is interpreted as representing a positive response.

Activation Assay

The aforementioned experiments were designed to minimise the response by quiescent or naive T-cells that can recognise RhD protein, but which are not activated by immunisation. To validate the experiments, the T-cells proliferated in the aforementioned experiment were tested using a modification of the method set out in European Journal of Immunology (1994) 24: 1578-1582 to identify if they had been activated *in vivo*. In this connection, the T-cells were screened to ascertain if they were from the subset bearing CD45RO which is a marker of previous activation or "memory", rather than from the subset bearing CD45RA which is the marker of quiescent or "naive" T-cells.

As shown in Figure 2 various peptide fragments have been selected in accordance with their particular peptide sequences. These are given in Tables 1, 2 and 3 which follow and the results achieved by means of the foregoing example are shown in Figure 2.

Accordingly we have shown that helper T-cells from all donors deliberately immunised against RhD can be stimulated *in vitro* by RhD peptides.

Further there is a variation between alloimmune donors in the T-cell response profile to the RhD peptides. Despite these variations, RhD peptides Nos. 2, 6, 6A, 10A, 11, 11A, 12, 12A, 15A, 18A, 28 and 39 are most commonly targeted and a proliferative response was elicited by peptide 12A in 70% of donors. However significantly related profiles are found in donors sharing HLA-DR alleles. It is predicted that alloreactive T-cell epitopes on the RhD protein would comprise sequences that are foreign to RhD-negative individuals, and would thus not be carried on the related RhCc/Ee protein that is expressed on the erythrocytes of such individuals. With the exception of peptide 28, all of the fragments identified are sequences that fulfil this prediction. It is therefore considered that such peptides, or derived sequences, could be used to stimulate either T-cell response or tolerance *in vivo* as desired, depending on the route of administration and/or the dose and formulation of the preparation.

The T-cells which were proliferated were in fact drawn from those that have been previously activated. This is important because it is these cells which will drive anti-D antibody production in RhD-negative donors immunised with RhD.

It follows that the characterisation of the putative helper T-cell epitopes we have identified is a key step in the development of safe immunogens for anti-immunoglobulin donors and opens the way to the evaluation of peptide immunotherapy as a novel approach to the prevention of haemolytic disease *inter alia* in neonates.

These experiments can be carried out using other rhesus proteins, such as RhC, Rhc, RhE or Rhe protein.

The aforementioned experiments were repeated using blood from subjects suffering from autoimmune haemolytic anaemia. It was therefore established that the T-cells of the subjects exhibited a proliferative response to peptides 2, 5, 14, 29, 31 and 38 (see Figure 3) and 65% of patients responded to peptide 29. The results also showed a correlation between patients suffering from autoimmune haemolytic anaemia and having tissue type HLA-DR15. With the exception of peptide 31 all of the peptides are shared in common between the RhD and RhCe/Ee proteins.

EXAMPLE 2

The HLA class II tissue type of the donors tested in Example 1 was ascertained by standard SSP-PCR methods. This was carried out because the molecules that determine tissue type select and bind antigenic peptide fragments for display to T-cells therefore they are important in this investigation.

The techniques described in Barker et al (1997)Blood 90:2701-2715 were used to determine that the HLA-D loci was more important than either the HLA-DP or HLA-DQ in the presentation of Rh D peptide fragments that stimulate T-cells *in vitro*.

A significant proportion of Rh D-negative donors selected for responsiveness to Rh D carry the HLA-DRB1*15 gene (56% versus approx. 29% in a control population). Thus carrying this tissue type is associated with an increase risk of producing anti-D antibodies after exposure to Rh D positive erythrocytes, and there is smaller variation in HLA-DR tissue type among responders than in the general population. It has also been shown that the patterns of Rh D peptides that

elicit T-cell proliferation are significantly related in Rh D-negative donors who share the same HLA-DR type (see Figures 3A and 3B).

For warm-type autoimmune haemolytic anaemia there is also an association with HLA DR15 with 65% of patients carrying this HLA type.

Nevertheless, a statistical analysis of all the data shows that the effect of HLA-DR type on the identity of the peptides recognised is relatively weak. In other words, many of the Rh D peptides stimulate T-cells regardless of tissue type.

These analyses demonstrate that the selection of Rh D peptide fragments for immunisation/tolerisation regimes may not be dependent on prior tissue typing of recipients, an important practical consideration for the clinical application of this approach.

EXAMPLE 3

Cultured T-cells are stimulated with each of the epitopes given in Tables 1 to 3 and after 5 days the responding cells were transferred to a flat-bottomed microtitre plates (96-well Nunc-Immuno Maxisorp) coated with 50μl per well of monoclonal anti-cytokine capture antibody diluted in 0.05M alkaline carbonate coating buffer pH 9.6. Unbound capture antibody was removed by two washes with HBSS and non-specific binding potential blocked by incubation with 200μl per well of phosphate buffered saline, pH 7.4 (PBS containing 3% BSA).

Five days after stimulation, lymphocyte cultures were mixed to resuspend the cells and duplicate 100μl aliquots were transferred into wells coated with the respective capture antibody specific for IFN-γ and or IL-10 or TGF-ß. The plates coated with capture antibodies and layered by lymphocytes were then incubated for a further 24 hours at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After this incubation the PBMC were removed by four washes with 0.2% Tween/PBS. One hundred microlitre aliquots of the appropriate biotinylated monoclonal detection antibody in 0.2% BSA/PBS were then added to the wells and incubated at room temperature for 45 minutes. After six washes with 0.5% Tween/PBS, 100μl of 1:100,000 ExtrAvidin-alkaline phosphatase conjugate (Sigma) was then added to each of the wells and incubated at room temperature for 30 minutes. The ExtrAvidin conjugate was removed by eight washes with 0.2% Tween/PBS, and the plates developed using 100μl per well of p-nitrophenyl phosphate (Sigma) 1.0mg/ml in 0.05M carbonate alkaline buffer pH 9.6. The absorbence of 405nm was then measured using a Multiscan plate reader (Labsystems Basingstoke UK).

Cytokine secretion was measured by interpolation from a standard curve generated by incubating duplicate wells with doubling dilutions of recombinant human IFN-γ or IL-10 or TGF-ß (Pharmingen). The standard curves were modelled by a smoothed cubic spline function applied to the absorbence reading and the cytokine concentrations after a quasilogarithmic transformation, where:

$$\text{quasilog}_e(z) = \log_e[z + \sqrt{[z^2 + 1]}).$$

This method is very sensitive and therefore can identify that a particular Rh D peptide is capable of stimulating human T-

cells to produce potentially immunosuppressive cytokines rather than to proliferate.

From Figures 5A and 5B it can be seen that epitopes 10, 16, 22, 24 and 34 induce IL-10 and/or TGF-β production by human T-cells. IL-10 and TGF-β molecules are known to have immunosuppressive properties. In preliminary experiments RhD peptides that induce IL-10 have been shown to inhibit T-cell proliferation in response to the entire RhD protein *in vitro*. Accordingly, prior administration of RhD peptides that elicit T-cell IL-10 or TGF-β production can be used to prevent RhD negative individuals from responding to RhD. It is also possible to inhibit established responses. This novel approach to manipulating the immune system has other application, including treatment of warm-type autoimmune haemolytic anaemia, in which the Rh proteins are important targets. The identification of peptides with similar properties derived from other antigens could also lead to therapy for a wide range of autoimmune diseases where the antigens/proteins are identified.

No IL-4 production was detected in any culture. In Figure 5C it can be seen that epitopes 5, 21 and 27 stimulate IFN-γ secretion. Figure 5D shows the level of incorporation of ^{3}H-Thymidine into the T-cells after stimulation with the RhD peptides.

From Figure 6 it can be seen that the addition of such peptides to T-cell cultures specifically blocks the proliferative response to the RhD protein, but not to a control antigen PPD. This result is very important since it raises the possibility that these peptides may also be able

to inhibit damaging responses *in vivo* if given to patients, whilst not suppressing the rest of the immune system.

TABLE 1

PEPTIDE NUMBER	PEPTIDE SEQUENCE	RESIDUES
RhCE (R2 cE)		
1	SSKYPRSVRRCLPLW	2 -16
2	CLPLWALTLEAALIL	12 -26
3	AALILLFYFFTHYDA	22 -36
4	THYDASLEDQKGLVA	32 -46
5	KGLVASYQVGQDLTV	42 -56
6	QDLTVMAALGLGFLT	52 -66
7	LGFLTSNFRRHSWSS	62 -76
8	HSWSSVAFNLFMLAL	72 -86
9	FMLALGVQWAILLDG	82 -96
10	ILLDGFLSQFPPGKV	92 -106
11	PPGKVVITLFSIRLA	102-116
12	SIRLATMSAMSVLIS	112-126
13	SVLISAGAVLGKVNL	122-136
14	GKVNLAQLVVMVLVE	132-146
15	MVLVEVTALGTLRMV	142-156
16	TLRMVISNIFNTDYH	152-166
17	NTDYHMNLRHFYVFA	162-176
18	FYVFAAYFGLTVAWC	172-186
19	TVAWCLPKPLPKGTE	182-196
20	PKGTEDNDQRATIPS	192-206
21	ATIPSLSAMLGALFL	202-216
22	GALFLWMFWPSVNSP	212-226
23	SVNSPLLRSPIQRKN	222-236
24	IQRKNAMFNTYYALA	232-246
25	YYALAVSVVTAISGS	242-256
26	AISGSSLAHPQRKIS	252-266
27	QRKISMTYVHSAVLA	262-276
28	SAVLAGGVAVGTSCH	272-286
29	GTSCHLIPSPWLAMV	282-296
30	WLAMVLGLVAGLISI	292-306
31	GLISIGGAKCLPVCC	302-316
32	LPVCCNRVLGIHHIS	312-326
33	IHHISVMHSIFSLLG	322-336
34	FSLLGLLGEITYIVL	332-346
35	TYIVLLVLHTVWNGN	342-356
36	VWNGNGMIGFQVLLS	352-366
37	QVLLSIGELSLAIVI	362-376
38	LAIVIALTSGLLTGL	372-386
39	LLTGLLLNLKIWKAP	382-396
40	IWKAPHVAKYFDDQV	392-406
41	FDDQVFWKFPHLAVG	402-416
42	DDQVFWKFPHLAVGF	403-417

TABLE 2

PEPTIDE NUMBER	PEPTIDE SEQUENCE	RESIDUES
RhCE (R1 Ce)		
1 (C)	SSKYPRSVRRCLPL**C**	2 -16
2 (C)	CLPL**C**ALTLEAALIL	12 -26
22 (e)	GALFLWMFWPSVNS**A**	212-226
23 (e)	SVNS**A**LLRSPIQRKN	222-236
RhD		
6 (also C)	QDLTVMAA**I**GLGFLT	52 -66
7 (also C)	LGFLTS**S**FRRHSWSS	62 -76
10 (also C)	ILLDGFLSQFP**S**GKV	92 -106
11 (also C)	P**S**GKVVITLFSIRLA	102-116
12	SIRLATMSA**L**SVLIS	112-126
13	SVLIS**VD**AVLGKVNL	122-136
15	MVLVEVTALG**N**LRMV	142-156
16	**N**LRMVISNIFNTDYH	152-166
17	NTDYHMN**MM**H**I**YVFA	162-176
18	**I**YVFAAYFGL**S**VAWC	172-186
19	**S**VAWCLPKPLP**E**GTE	182-196
20	P**E**GTED**K**DQ**T**ATIPS	192-206
22	GALFLW**I**FWPS**F**NS**A**	212-226
23	S**F**NS**A**LLRSPI**E**RKN	222-236
24	I**E**RKNA**V**FNTYYA**VA**	232-246
25	YYA**V**AVSVVTAISGS	242-256
26	AISGSSLAHPQ**G**KIS	252-266
27	Q**G**KIS**K**TYVHSAVLA	262-276
30	WLAMVLGLVAGLIS**V**	292-306
31	GLIS**V**GGAK**Y**LP**G**CC	302-316
32	LP**G**CCNRVLGI**P**H**S**S	312-326
33	I**P**H**S**SIM**GYN**FSLLG	322-336
34	FSLLGLLGEI**I**YIVL	332-346
35	**I**YIVLLVL**D**TV**GA**GN	342-356
36	V**GA**GNGMIGFQVLLS	352-366
40	IWKAPH**E**AKYFDDQV	392-406

TABLE 3

PEPTIDE NUMBER	PEPTIDE SEQUENCE	RESIDUES
RhCE (R1 Ce)		
1A (C)	RSVRRCLPLCALTLE	7 -21
22A(e)	WMFWPSVNSALLRSP	217-231
RhD		
6A (also C)	MAAIGLGFLTSSFRR	57 -71
7A (also C)	SSFRRHSWSSVAFNL	67 -81
10A(also C)	FLSQFPSGKVVITLF	97 -111
11A(also C)	VITLFSIRLATMSAL	107-121
12A	TMSALSVLISVDAVL	117-131
13A	VDAVLGKVNLAQLVV	127-141
15A	VTALGNLRMVISNIF	147-161
16A	ISNIFNTDYHMNMMH	157-171
17A	MNMMHIYVFAAYFGL	167-181
18A	AYFGLSVAWCLPKPL	177-191
19A	LPKPLPEGTEDKDQT	187-201
20A	DKDQTATIPSLSAML	197-211
21A	LSAMLGALFLWIFWP	207-221
22A	WIFWPSFNSALLRSP	217-231
23A	LLRSPIERKNAVFNT	227-241
24A	AVFNTYYAVAVSVVT	237-251
26A	SLAHPQGKISKTYVH	257-271
27A	KTYVHSAVLAGGVAV	267-281
30A	LGLVAGLISVGGAKY	297-311
31A	GGAKYLPGCCNRVLG	307-321
32A	NRVLGIPHSSIMGYN	317-331
33A	IMGYNFSLLGLLGEI	327-341
34A	LLGEIIYIVLLVLDT	337-351
35A	LVLDTVGAGNGMIGF	347-361
39A	LLNLKIWKAPHEAKY	387-401
40A	HEAKYFDDQVFWKFP	397-411

TABLE 4

PEPTIDE NUMBER	PEPTIDE SEQUENCE	RESIDUES
Rh50 GP		
1	MRFTFPLMAIVLEIA	1 -15
2	VLEIAMIVLFGLFVE	11 -25
3	GLFVEYETDQTVLEQ	21 -35
4	TVLEQLNITKPTDMG	31 -45
5	PTDMGIFFELYPLFQ	41 -55
6	YPLFQDVHVMIFVGF	51 -65
7	IFVGFGFLMTFLKKY	61 -75
8	FLKKYGFSSVGINLL	71 -85
9	GINLLVAALGLQWGT	81 -95
10	LQWGTIVQGILQSQG	91 -105
11	LQSQGQKFNIGIKNM	101-115
12	GIKNMINADFSAATV	111-125
13	SAATVLISFGAVLGK	121-135
14	AVLGKTSPTQMLIMT	131-145
15	MLIMTILEIVFFAHN	141-155
16	FFAHNEYLVSEIFKA	151-165
17	EIFKASDIGASMTIH	161-175
18	SMTIHAFGAYFGLAV	171-185
19	FGLAVAGILYRSGLR	181-195
20	RSGLRKGHENEESAY	191-205
21	EESAYYSDLFAMIGT	201-215
22	AMIGTLFLWMFWPSF	211-225
23	FWPSFNSAIAEPGDK	221-235
24	EPGDKQCRAIVDTYF	231-245
25	VDTYFSLAACVLTAF	241-255
26	VLTAFAFSSLVEHRG	251-265
27	VEHRGKLNMVHIQNA	261-275
28	HIQNATLAGGVAVGT	271-285
29	VAVGTCADMAIHPFG	281-295
30	IHPFGSMIIGSIAGM	291-305
31	SIAGMVSVLGYKFLT	301-315
32	YKFLTPLFTTKLRIH	311-325
33	KLRIHDTCGVHNLHG	321-335
34	HNLHGLPGVVGGLAG	331-345
35	GGLAGIVAVAMGASN	341-355
36	MGASNTSMAMQAAAL	351-365
37	QAAALGSSIGTAVVG	361-375
38	TAVVGGLMTGLILKL	371-385
39	LILKLPLWGQPSDQN	381-395
40	PSDQNCYDDSVYWKV	391-405
41	NCYDDSVYWKVPKTR	395-409
Other Peptides		
BR	SKYPNCAYKTTQANKH	
AV2	TIPEQSFQGSPSADT	
AV4	TVKADFEFSSAPAPD	
AV6	TVEERQQFGELPVSE	
P23	ELKIISRCQVCMKKRH	
HA	PKYVKQNTLKLAT	

CLAIMS:-

1. A pharmaceutical composition for the prevention of alloimmunisation of a subject, said composition comprising an immunologically effective epitope of a rhesus protein or an immunologically active analogue or derivative thereof.

2. A pharmaceutical composition for the immunosuppression of a response elicited by alloimmunisation of a subject or an autoimmune haemolytic disease, said composition comprising an immunologically effective epitope of a rhesus protein or an immunologically active analogue or derivative thereof.

3. A pharmaceutical composition according to claim 2 wherein the autoimmune disease is idiopathic or secondary autoimmune haemolytic anaemia mediated by 'warm-type' antibodies.

4. A pharmaceutical composition according to any preceding claim wherein the rhesus protein is either RhD, RhC, Rhc, RhE or Rhe protein.

5. A pharmaceutical composition according to claim 4 comprising an epitope selected from at least one of numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth.

6. A pharmaceutical composition according to either claims 4 or 5 wherein the epitope is either epitope 12A when alloimmunisation has occurred; or epitope 29 for autoimmune haemolytic anaemia.

7. A pharmaceutical composition according to any preceding claim wherein the epitope or immunoreactive derivative is synthesised.

8. A pharmaceutical composition for the induction of alloimmunisation of a subject, said composition comprising an immunologically effective epitope of a rhesus protein or an immunologically active analogue or derivative thereof disposed in a pharmacologically acceptable vehicle.

9. A pharmaceutical composition according to claim 8 wherein the rhesus protein is either RhD, RhC, Rhc, RhE or Rhe protein.

10. A pharmaceutical composition according to claim 9 comprising an epitope selected from at least one of numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth.

11. A pharmaceutical composition according to either claim 9 or 10 wherein the epitope is either epitope 12A when alloimmunisation has occurred; or epitope 29 for autoimmune haemolytic anaemia.

12. A pharmaceutical composition according to any of claims 8 to 11 wherein the vehicle is selected such that the composition is in an injectable, oral, rectal, topical or spray-uptake form.

13. A tolerising peptide fragment disposed in a pharmacologically effective vehicle, said vehicle being adapted for injection oral, rectal, topical or spray-uptake

administration to the subject wherein the peptide fragment is an epitope of either a RhD, RhC, Rhc, RhE or Rhe protein.

14. A tolerising peptide fragment according to claim 13 selected from at least one of an epitope numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth.

15. A tolerising peptide fragment according to either claim 13 or 14 wherein the fragment is either epitope 12A when alloimmunisation has occurred; or 29 for autoimmune haemolytic anaemia.

16. A tolerising peptide fragment according to any of claims 13 to 15 wherein the pharmaceutically acceptable vehicle is adapted for transdermal or transmucosal administration or wherein said vehicle is a formulation with an enteric coating for oral administration.

17. A method of tolerising a subject which comprises administering to said subject a tolerising peptide fragment according to any one of claims 13 to 16.

18. An epitope from a RhD, RhC, Rhc, RhE or Rhe protein selected from at least one of epitope numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth.

19. The use in the manufacture of a medicament for the tolerisation of a patient who may become alloimmunised comprising an epitope selected from a RhD, RhC, Rhc, RhE or Rhe protein or selected from at least one of epitope numbers

2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth, and a pharmaceutically acceptable vehicle therefor.

20. The use in the manufacture of a medicament for the immunosuppression of an alloimmunised patient or a patient with warm-type autoimmune haemolytic anaemia comprising an epitope selected from a RhD, RhC, Rhc, RhE or Rhe protein or selected from at least one of epitope numbers 2, 5, 6, 6A, 10A, 11, 11A, 12, 12A, 14, 15A, 18A, 28, 29, 31, 38 and 39 hereinbefore set forth and a pharmaceutically acceptable vehicle therefor.

21. The use according to either claim 19 or 20 wherein the vehicle is adapted for transdermal or transmucosal administration.

22. A method for determining effect of one or more epitopes from a rhesus protein on a human lymphocyte, *in vitro*, comprising:-

a) stimulating the lymphocyte with one or more epitope/ peptide of a rhesus protein;

b) between 4 and 7 days later resuspending the cultures and transferring aliquots into plates prepared in the following manner;

c) coating each well in the plate with monoclonal anti-cytokine capture antibody;

d) washing the plate at least once with Hanks Buffered Salt Solution (HBSS);

e) blocking any non-specific binding using an appropriate solution;

f) incubating the plates with the lymphocyte culture for 12-36 hours at 30-40°C in an atmosphere of substantially 5% CO_2 and substantially 95% air;

g) washing the plates at least once with Tween/PBS;

h) introducing an appropriate biotinylates monoclonal detection antibody to each well and incubating for 30-60 mins at room temperature;

i) washing the plates at least once with Tween/PBS;

j) introducing of ExtrAvidin-alkaline phosphatase conjugate and incubating for 15-45mins;

k) washing the plates at least once with Tween/PBS;

l) developing the plates with 50-150µl per well of p-nitrophenyl phosphate in 0.05M carbonate alkaline buffer pH9.6 added to each well;

m) reading the absorbence at 405nm.

23. A method for the determination of the propensity of a RhD negative subject to produce anti-D antibodies after exposure to Rh D positive blood comprising ascertaining the tissue type of the subject and determining if it is positive for HLA-DRB1*15.

```
RHC: MSSKYPRSVR RCLPLCALTL EAALILLFYF FTHYDASLED QKGLVASYQV  50
RHc:              W
RHD:              W
RHC: GQDLTVMAAI GLGFLTSSFR RHSWSSVAFN LFMLALGVQW AILLDGFLSQ 100
RHc:            L     N
RHD:            I     S
RHC: FPSGKVVITL FSIRLATMSA MSVLISAGAV LGKVNLAQLV VMVLVEVTAL 150
RHc:    P
RHD:    S                       L     VD
RHC: GTLRMVISNI FNTDYHMNLR HFYVFAAYFG LTVAWCLPKP LPKGTEDNDQ 200
RHD: N                       MM  I       S           E
RHE: RATIPSLSAM LGALFLWMFW PSVNSPLLRS PIQRKNAMFN TYYALAVSVV 250
RHe:                                 A
RHD: T                   I     F  A       E     V           V
RHC: TAISGSSLAH PQRKISMTYV HSAVLAGGVA VGTSCHLIPS PWLAMVLGLV 300
RHD:                     G     K
RHC: AGLISIGGAK CLPVCCNRVL GIHHISVMHS IFSLLGLLGE ITYIVLLVLH 350
RHD:          V     Y  G         P S I GY N          I          D
RHC: TVWNGNGMIG FQVLLSIGEL SLAIVIALTS GLLTGLLLNL KIWKAPHVAK 400
RHD:    GA                                                    E
RHC: YFDDQVFWKF PHLAVGF
RHD:
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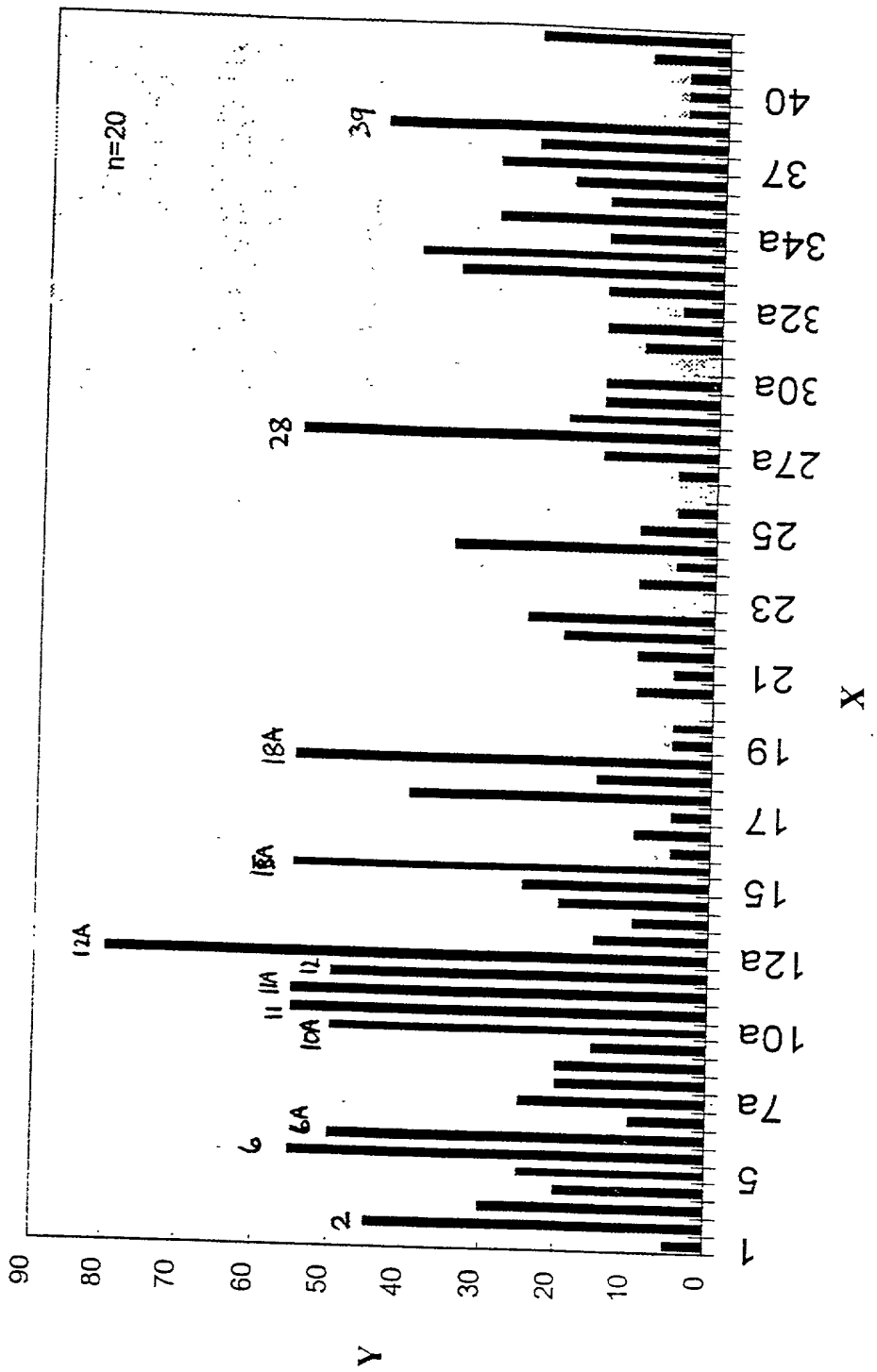
Figure 1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39

Figure 2

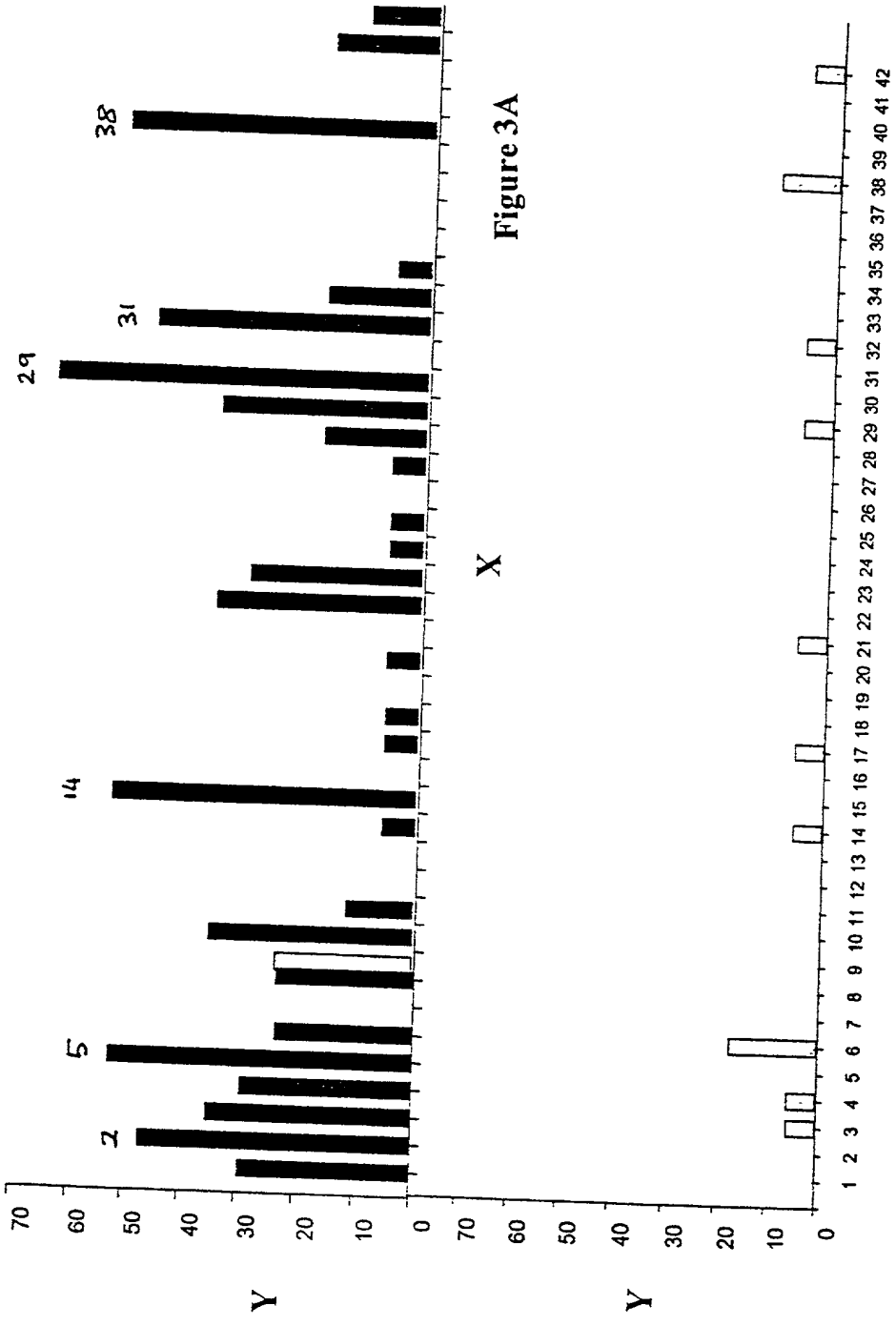

Figure 3A

Figure 3B

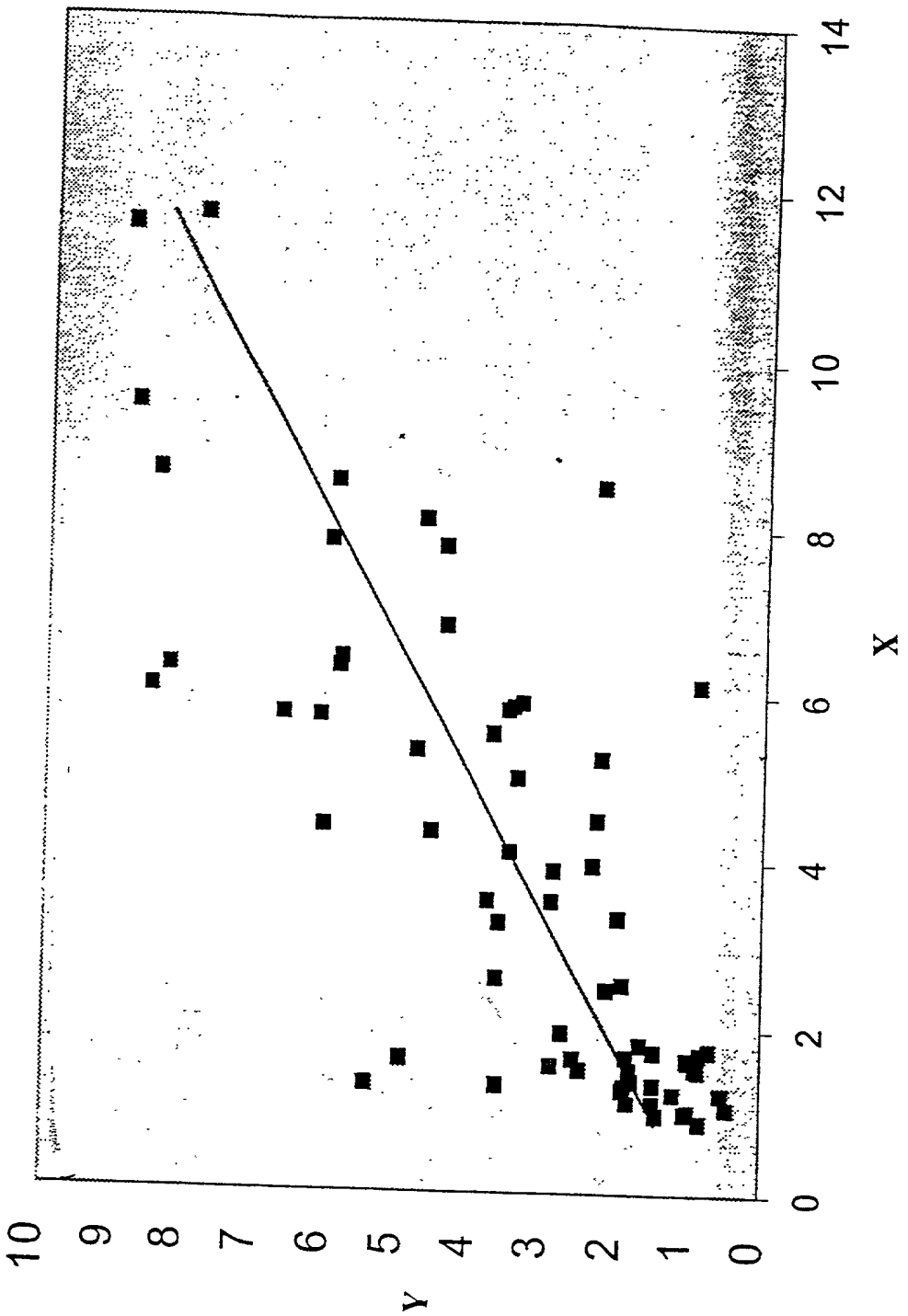

Figure 4

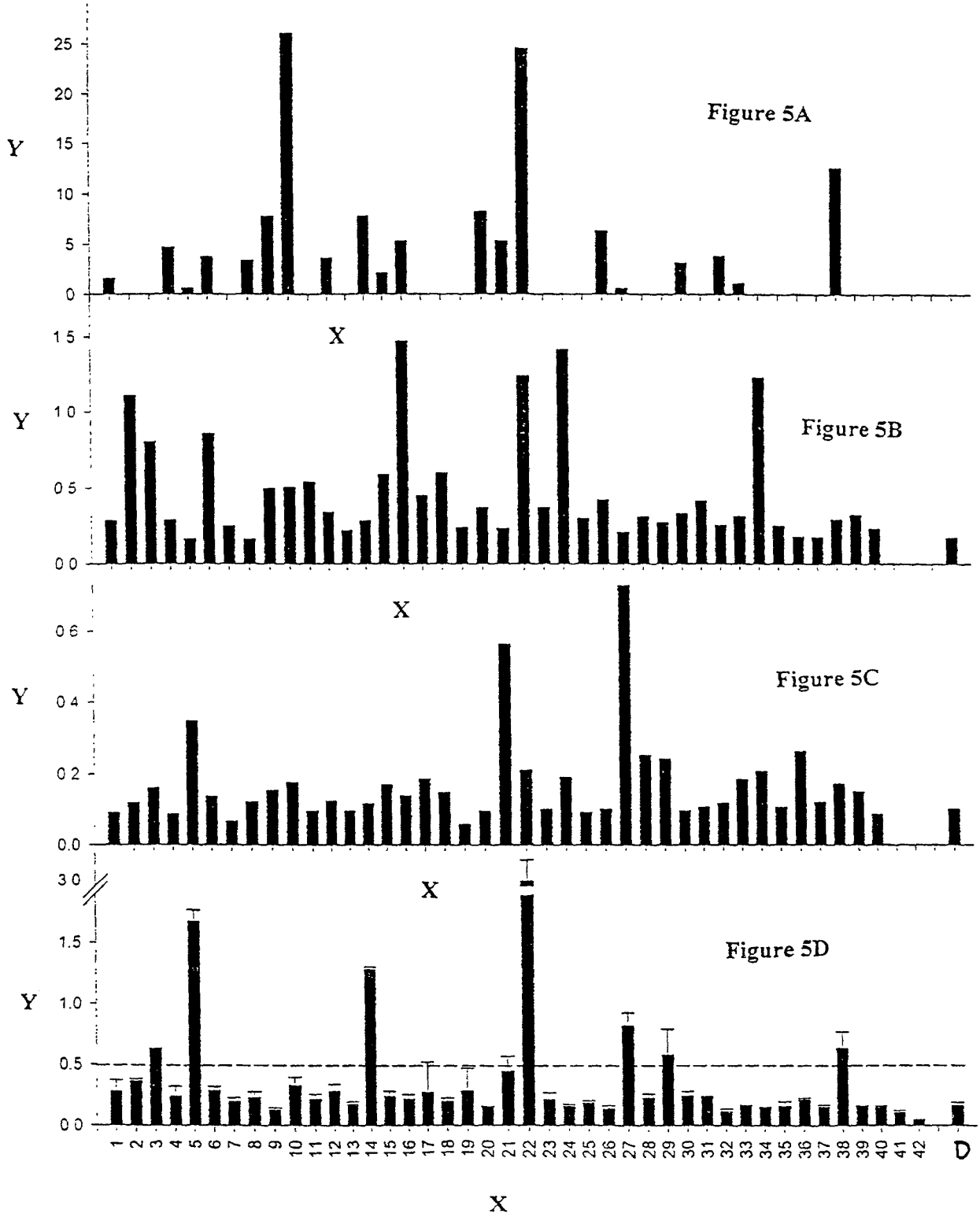
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Figure 5A
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0 4
0 2
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X
Figure 5C
Y
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1.5
1.0
0.5
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Figure 5D
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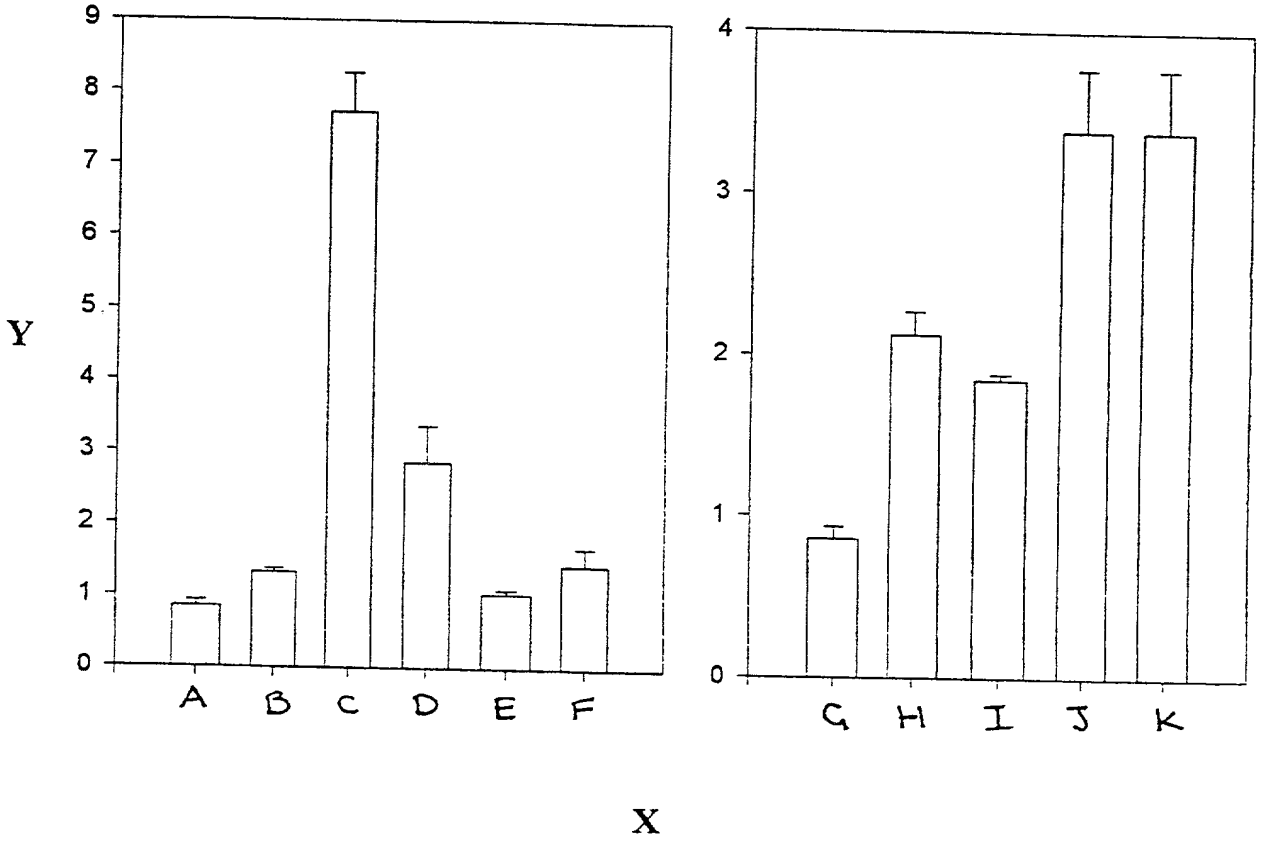

Figure 6

Docket No.
ABLE-0020

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Allo and Auto-Reactive T-Cell Epitopes

☐ the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **1 December 1999** as United States Application No. or PCT International Application Number **PCT/GB99/04027**

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
9826378.3	**Great Britain**	**1 December 1998**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

26259

PATENT TRADEMARK OFFICE

☐ Send Correspondence to:

Direct Telephone Calls to: *(name and telephone number)*
Jane Massey Licata or Kathleen A. Tyrrell - (856) 810-1515

Full name of sole or first inventor **Stanislaw Joseph Urbaniak**	
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SEQUENCE LISTING

<110> Aberdeen University
The Common Servics Agency For The Scottish Health

<120> ALLO- AND AUTO-REACTIVE T-CELL EPITOPES

<130> P097

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<141> 1999-12-01

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Cys Leu Pro Leu Trp Ala Leu Thr Leu Glu Ala Ala Leu Ile Leu
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Ala Ala Leu Ile Leu Leu Phe Tyr Phe Phe Thr His Tyr Asp Ala
1 5 10 15

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Thr His Tyr Asp Ala Ser Leu Glu Asp Gln Lys Gly Leu Val Ala
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Lys Gly Leu Val Ala Ser Tyr Gln Val Gly Gln Asp Leu Thr Val
1 5 10 15

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Gln Asp Leu Thr Val Met Ala Ala Leu Gly Leu Gly Phe Leu Thr
1 5 10 15

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Leu Gly Phe Leu Thr Ser Asn Phe Arg Arg His Ser Trp Ser Ser
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His Ser Trp Ser Ser Val Ala Phe Asn Leu Phe Met Leu Ala Leu
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1 5 10 15

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Pro Pro Gly Lys Val Val Ile Thr Leu Phe Ser Ile Arg Leu Ala
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Phe Tyr Val Phe Ala Ala Tyr Phe Gly Leu Thr Val Ala Trp Cys
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Ile Gln Arg Lys Asn Ala Met Phe Asn Thr Tyr Tyr Ala Leu Ala
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Tyr Tyr Ala Leu Ala Val Ser Val Val Thr Ala Ile Ser Gly Ser
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Gln Arg Lys Ile Ser Met Thr Tyr Val His Ser Ala Val Leu Ala
1 5 10 15

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<400> 29
Gly Thr Ser Cys His Leu Ile Pro Ser Pro Trp Leu Ala Met Val
1 5 10 15

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Trp Leu Ala Met Val Leu Gly Leu Val Ala Gly Leu Ile Ser Ile
1 5 10 15

<210> 31
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<223> Residue 302-316

<400> 31
Gly Leu Ile Ser Ile Gly Gly Ala Lys Cys Leu Pro Val Cys Cys
1 5 10 15

<210> 32
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<213> RhCE (R2 CE)

<220>
<223> Residue 312-326

<400> 32
Leu Pro Val Cys Cys Asn Arg Val Leu Gly Ile His His Ile Ser
1 5 10 15

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Ile His His Ile Ser Val Met His Ser Ile Phe Ser Leu Leu Gly
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<220>
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Phe Ser Leu Leu Gly Leu Leu Gly Glu Ile Thr Tyr Ile Val Leu
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Thr Tyr Ile Val Leu Leu Val Leu His Thr Val Trp Asn Gly Asn
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Val Trp Asn Gly Asn Gly Met Ile Gly Phe Gln Val Leu Leu Ser
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<400> 37
Gln Val Leu Leu Ser Ile Gly Glu Leu Ser Leu Ala Ile Val Ile
1 5 10 15

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<400> 38
Leu Ala Ile Val Ile Ala Leu Thr Ser Gly Leu Leu Thr Gly Leu
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Asp Asp Gln Val Phe Trp Lys Phe Pro His Leu Ala Val Gly Phe
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Ser Ser Lys Tyr Pro Arg Ser Val Arg Arg Cys Leu Pro Leu Cys
1 5 10 15

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<220>
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Cys Leu Pro Leu Cys Ala Leu Thr Leu Glu Ala Ala Leu Ile Leu
1 5 10 15

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Gly Ala Leu Phe Leu Trp Met Phe Trp Pro Ser Val Asn Ser Ala
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Ser Val Asn Ser Ala Leu Leu Arg Ser Pro Ile Gln Arg Lys Asn
1 5 10 15

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<400> 47

Gln Asp Leu Thr Val Met Ala Ala Ile Gly Leu Gly Phe Leu Thr
1 5 10 15

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<400> 48
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Pro Ser Gly Lys Val Val Ile Thr Leu Phe Ser Ile Arg Leu Ala
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Ser Val Leu Ile Ser Val Asp Ala Val Leu Gly Lys Val Asn Leu
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Met Val Leu Val Glu Val Thr Ala Leu Gly Asn Leu Arg Met Val
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1 5 10 15

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Ser Val Ala Trp Cys Leu Pro Lys Pro Leu Pro Glu Gly Thr Glu
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Pro Glu Gly Thr Glu Asp Lys Asp Gln Thr Ala Thr Ile Pro Ser
1 5 10 15

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<400> 59
Gly Ala Leu Phe Leu Trp Ile Phe Trp Pro Ser Phe Asn Ser Ala
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Ile Glu Arg Lys Asn Ala Val Phe Asn Thr Tyr Tyr Ala Val Ala
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<400> 62
Tyr Tyr Ala Val Ala Val Ser Val Val Thr Ala Ile Ser Gly Ser
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<400> 63
Ala Ile Ser Gly Ser Ser Leu Ala His Pro Gln Gly Lys Ile Ser
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<400> 64
Gln Gly Lys Ile Ser Lys Thr Tyr Val His Ser Ala Val Leu Ala
1 5 10 15

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<400> 65
Trp Leu Ala Met Val Leu Gly Leu Val Ala Gly Leu Ile Ser Val
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Gly Leu Ile Ser Val Gly Gly Ala Lys Tyr Leu Pro Gly Cys Cys
1 5 10 15

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Leu Pro Gly Cys Cys Asn Arg Val Leu Gly Ile Pro His Ser Ser
1 5 10 15

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<400> 68
Ile Pro His Ser Ser Ile Met Gly Tyr Asn Phe Ser Leu Leu Gly
1 5 10 15

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<400> 69
Phe Ser Leu Leu Gly Leu Leu Gly Glu Ile Ile Tyr Ile Val Leu
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Ile Tyr Ile Val Leu Leu Val Leu Asp Thr Val Gly Ala Gly Asn
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<400> 71

Val Gly Ala Gly Asn Gly Met Ile Gly Phe Gln Val Leu Leu Ser
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<400> 72
Ile Trp Lys Ala Pro His Glu Ala Lys Tyr Phe Asp Asp Gln Val
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Arg Ser Val Arg Arg Cys Leu Pro Leu Cys Ala Leu Thr Leu Glu
1 5 10 15

<210> 74
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Trp Met Phe Trp Pro Ser Val Asn Ser Ala Leu Leu Arg Ser Pro
1 5 10 15

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<220>
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Met Ala Ala Ile Gly Leu Gly Phe Leu Thr Ser Ser Phe Arg Arg
1 5 10 15

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<400> 76
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Phe Leu Ser Gln Phe Pro Ser Gly Lys Val Val Ile Thr Leu Phe
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<400> 78
Val Ile Thr Leu Phe Ser Ile Arg Leu Ala Thr Met Ser Ala Leu
1 5 10 15

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<400> 79
Thr Met Ser Ala Leu Ser Val Leu Ile Ser Val Asp Ala Val Leu
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<400> 80
Val Asp Ala Val Leu Gly Lys Val Asn Leu Ala Gln Leu Val Val
1 5 10 15

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Val Thr Ala Leu Gly Asn Leu Arg Met Val Ile Ser Asn Ile Phe
1 5 10 15

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<400> 82
Ile Ser Asn Ile Phe Asn Thr Asp Tyr His Met Asn Met Met His
1 5 10 15

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Met Asn Met Met His Ile Tyr Val Phe Ala Ala Tyr Phe Gly Leu
1 5 10 15

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1 5 10 15

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Leu Pro Lys Pro Leu Pro Glu Gly Thr Glu Asp Lys Asp Gln Thr
1 5 10 15

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Asp Lys Asp Gln Thr Ala Thr Ile Pro Ser Leu Ser Ala Met Leu
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<400> 88
Trp Ile Phe Trp Pro Ser Phe Asn Ser Ala Leu Leu Arg Ser Pro
1 5 10 15

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<400> 89
Leu Leu Arg Ser Pro Ile Glu Arg Lys Asn Ala Val Phe Asn Thr
1 5 10 15

<210> 90
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<400> 90
Ala Val Phe Asn Thr Tyr Tyr Ala Val Ala Val Ser Val Val Thr
1 5 10 15

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<220>
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<400> 91
Ser Leu Ala His Pro Gln Gly Lys Ile Ser Lys Thr Tyr Val His
1 5 10 15

<210> 92
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<220>
<223> Residue 267-281

<400> 92
Lys Thr Tyr Val His Ser Ala Val Leu Ala Gly Gly Val Ala Val
1 5 10 15

<210> 93
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<220>
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<400> 93
Leu Gly Leu Val Ala Gly Leu Ile Ser Val Gly Gly Ala Lys Tyr
1 5 10 15

<210> 94
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<400> 94
Gly Gly Ala Lys Tyr Leu Pro Gly Cys Cys Asn Arg Val Leu Gly
1 5 10 15

<210> 95
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<400> 95

Asn Arg Val Leu Gly Ile Pro His Ser Ser Ile Met Gly Tyr Asn
1 5 10 15

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<400> 96
Ile Met Gly Tyr Asn Phe Ser Leu Leu Gly Leu Leu Gly Glu Ile
1 5 10 15

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<400> 97
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1 5 10 15

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<400> 98
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1 5 10 15

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<220>
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1 5 10 15

<210> 102
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<400> 102
Val Leu Glu Ile Ala Met Ile Val Leu Phe Gly Leu Phe Val Glu
1 5 10 15

<210> 103
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<400> 103
Gly Leu Phe Val Glu Tyr Glu Thr Asp Gln Thr Val Leu Glu Gln
1 5 10 15

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<400> 104
Thr Val Leu Glu Gln Leu Asn Ile Thr Lys Pro Thr Asp Met Gly
1 5 10 15

<210> 105
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<212> PRT
<213> Rh50 GP

<220>
<223> Residue 41-55

<400> 105
Pro Thr Asp Met Gly Ile Phe Phe Glu Leu Tyr Pro Leu Phe Gln
1 5 10 15

<210> 106
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 51-65

<400> 106
Tyr Pro Leu Phe Gln Asp Val His Val Met Ile Phe Val Gly Phe
1 5 10 15

<210> 107
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 61-75

<400> 107
Ile Phe Val Gly Phe Gly Phe Leu Met Thr Phe Leu Lys Lys Tyr
1 5 10 15

<210> 108
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 71-85

<400> 108
Phe Leu Lys Lys Tyr Gly Phe Ser Ser Val Gly Ile Asn Leu Leu
1 5 10 15

<210> 109
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 81-95

<400> 109
Gly Ile Asn Leu Leu Val Ala Ala Leu Gly Leu Gln Trp Gly Thr
1 5 10 15

<210> 110
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 91-105

<400> 110
Leu Gln Trp Gly Thr Ile Val Gln Gly Ile Leu Gln Ser Gln Gly
1 5 10 15

<210> 111
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 101-115

<400> 111
Leu Gln Ser Gln Gly Gln Lys Phe Asn Ile Gly Ile Lys Asn Met
1 5 10 15

<210> 112
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 111-125

<400> 112
Gly Ile Lys Asn Met Ile Asn Ala Asp Phe Ser Ala Ala Thr Val
1 5 10 15

<210> 113
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 121-135

<400> 113
Ser Ala Ala Thr Val Leu Ile Ser Phe Gly Ala Val Leu Gly Lys
1 5 10 15

<210> 114
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 131-145

<400> 114
Ala Val Leu Gly Lys Thr Ser Pro Thr Gln Met Leu Ile Met Thr
1 5 10 15

<210> 115
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 141-155

<400> 115
Met Leu Ile Met Thr Ile Leu Glu Ile Val Phe Phe Ala His Asn
1 5 10 15

<210> 116
<211> 15
<212> PRT

<213> Rh50 GP

<220>
<223> Residue 151-165

<400> 116
Phe Phe Ala His Asn Glu Tyr Leu Val Ser Glu Ile Phe Lys Ala
1 5 10 15

<210> 117
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 161-175

<400> 117
Glu Ile Phe Lys Ala Ser Asp Ile Gly Ala Ser Met Thr Ile His
1 5 10 15

<210> 118
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 171-185

<400> 118
Ser Met Thr Ile His Ala Phe Gly Ala Tyr Phe Gly Leu Ala Val
1 5 10 15

<210> 119
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 181-195

<400> 119

Phe Gly Leu Ala Val Ala Gly Ile Leu Tyr Arg Ser Gly Leu Arg
1 5 10 15

<210> 120
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 191-205

<400> 120
Arg Ser Gly Leu Arg Lys Gly His Glu Asn Glu Glu Ser Ala Tyr
1 5 10 15

<210> 121
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 201-215

<400> 121
Glu Glu Ser Ala Tyr Tyr Ser Asp Leu Phe Ala Met Ile Gly Thr
1 5 10 15

<210> 122
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 211-225

<400> 122
Ala Met Ile Gly Thr Leu Phe Leu Trp Met Phe Trp Pro Ser Phe
1 5 10 15

<210> 123

<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 221-235

<400> 123
Phe Trp Pro Ser Phe Asn Ser Ala Ile Ala Glu Pro Gly Asp Lys
1 5 10 15

<210> 124
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 231-245

<400> 124
Glu Pro Gly Asp Lys Gln Cys Arg Ala Ile Val Asp Thr Tyr Phe
1 5 10 15

<210> 125
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 241-255

<400> 125
Val Asp Thr Tyr Phe Ser Leu Ala Ala Cys Val Leu Thr Ala Phe
1 5 10 15

<210> 126
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 251-265

<400> 126
Val Leu Thr Ala Phe Ala Phe Ser Ser Leu Val Glu His Arg Gly
1 5 10 15

<210> 127
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 261-275

<400> 127
Val Glu His Arg Gly Lys Leu Asn Met Val His Ile Gln Asn Ala
1 5 10 15

<210> 128
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 271-285

<400> 128
His Ile Gln Asn Ala Thr Leu Ala Gly Gly Val Ala Val Gly Thr
1 5 10 15

<210> 129
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 281-295

<400> 129
Val Ala Val Gly Thr Cys Ala Asp Met Ala Ile His Pro Phe Gly
1 5 10 15

<210> 130
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 291-305

<400> 130
Ile His Pro Phe Gly Ser Met Ile Ile Gly Ser Ile Ala Gly Met
1 5 10 15

<210> 131
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 301-315

<400> 131
Ser Ile Ala Gly Met Val Ser Val Leu Gly Tyr Lys Phe Leu Thr
1 5 10 15

<210> 132
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 311-325

<400> 132
Tyr Lys Phe Leu Thr Pro Leu Phe Thr Thr Lys Leu Arg Ile His
1 5 10 15

<210> 133
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 321-335

<400> 133
Lys Leu Arg Ile His Asp Thr Cys Gly Val His Asn Leu His Gly
1 5 10 15

<210> 134
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 331-345

<400> 134
His Asn Leu His Gly Leu Pro Gly Val Val Gly Gly Leu Ala Gly
1 5 10 15

<210> 135
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 341-355

<400> 135
Gly Gly Leu Ala Gly Ile Val Ala Val Ala Met Gly Ala Ser Asn
1 5 10 15

<210> 136
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 351-365

<400> 136
Met Gly Ala Ser Asn Thr Ser Met Ala Met Gln Ala Ala Ala Leu
1 5 10 15

<210> 137
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 361-375

<400> 137
Gln Ala Ala Ala Leu Gly Ser Ser Ile Gly Thr Ala Val Val Gly
1 5 10 15

<210> 138
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 371-385

<400> 138
Thr Ala Val Val Gly Gly Leu Met Thr Gly Leu Ile Leu Lys Leu
1 5 10 15

<210> 139
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 381-395

<400> 139
Leu Ile Leu Lys Leu Pro Leu Trp Gly Gln Pro Ser Asp Gln Asn
1 5 10 15

<210> 140
<211> 15
<212> PRT

<213> Rh50 GP

<220>
<223> Residue 391-405

<400> 140
Pro Ser Asp Gln Asn Cys Tyr Asp Asp Ser Val Tyr Trp Lys Val
1 5 10 15

<210> 141
<211> 15
<212> PRT
<213> Rh50 GP

<220>
<223> Residue 395-409

<400> 141
Asn Cys Tyr Asp Asp Ser Val Tyr Trp Lys Val Pro Lys Thr Arg
1 5 10 15

<210> 142
<211> 16
<212> PRT
<213> BR

<400> 142
Ser Lys Tyr Pro Asn Cys Ala Tyr Lys Thr Thr Gln Ala Asn Lys His
1 5 10 15

<210> 143
<211> 15
<212> PRT
<213> AV2

<400> 143
Thr Ile Pro Glu Gln Ser Phe Gln Gly Ser Pro Ser Ala Asp Thr
1 5 10 15

<210> 144

<211> 15
<212> PRT
<213> AV4

<400> 144
Thr Val Lys Ala Asp Phe Glu Phe Ser Ser Ala Pro Ala Pro Asp
1 5 10 15

<210> 145
<211> 15
<212> PRT
<213> AV6

<400> 145
Thr Val Glu Glu Arg Gln Gln Phe Gly Glu Leu Pro Val Ser Glu
1 5 10 15

<210> 146
<211> 16
<212> PRT
<213> P23

<400> 146
Glu Leu Lys Ile Ile Ser Arg Cys Gln Val Cys Met Lys Lys Arg His
1 5 10 15

<210> 147
<211> 13
<212> PRT
<213> HA

<400> 147
Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr
1 5 10

<210> 148
<211> 417
<212> PRT
<213> RhCE

<220>
<223> Residue 111-125

<400> 148

```
Met Ser Ser Lys Tyr Pro Arg Ser Val Arg Arg Cys Leu Pro Leu Cys
 1               5                  10                  15

Ala Leu Thr Leu Glu Ala Ala Leu Ile Leu Leu Phe Tyr Phe Phe Thr
            20                  25                  30

His Tyr Asp Ala Ser Leu Glu Asp Gln Lys Gly Leu Val Ala Ser Tyr
        35                  40                  45

Gln Val Gly Gln Asp Leu Thr Val Met Ala Ala Ile Gly Leu Gly Phe
    50                  55                  60

Leu Thr Ser Ser Phe Arg Arg His Ser Trp Ser Ser Val Ala Phe Asn
65                  70                  75                  80

Leu Phe Met Leu Ala Leu Gly Val Gln Trp Ala Ile Leu Leu Asp Gly
                85                  90                  95

Phe Leu Ser Gln Phe Pro Ser Gly Lys Val Val Ile Thr Leu Phe Ser
            100                 105                 110

Ile Arg Leu Ala Thr Met Ser Ala Met Ser Val Leu Ile Ser Ala Gly
        115                 120                 125

Ala Val Leu Gly Lys Val Asn Leu Ala Gln Leu Val Val Met Val Leu
    130                 135                 140

Val Glu Val Thr Ala Leu Gly Thr Leu Arg Met Val Ile Ser Asn Ile
145                 150                 155                 160

Phe Asn Thr Asp Tyr His Met Asn Leu Arg His Phe Tyr Val Phe Ala
                165                 170                 175

Ala Tyr Phe Gly Leu Thr Val Ala Trp Cys Leu Pro Lys Pro Leu Pro
            180                 185                 190

Lys Gly Thr Glu Asp Asn Asp Gln Arg Ala Thr Ile Pro Ser Leu Ser
        195                 200                 205

Ala Met Leu Gly Ala Leu Phe Leu Trp Met Phe Trp Pro Ser Val Asn
    210                 215                 220

Ser Pro Leu Leu Arg Ser Pro Ile Gln Arg Lys Asn Ala Met Phe Asn
225                 230                 235                 240
```

```
Thr Tyr Tyr Ala Leu Ala Val Ser Val Val Thr Ala Ile Ser Gly Ser
                245                 250                 255

Ser Leu Ala His Pro Gln Arg Lys Ile Ser Met Thr Tyr Val His Ser
            260                 265                 270

Ala Val Leu Ala Gly Gly Val Ala Val Gly Thr Ser Cys His Leu Ile
        275                 280                 285

Pro Ser Pro Trp Leu Ala Met Val Leu Gly Leu Val Ala Gly Leu Ile
    290                 295                 300

Ser Ile Gly Gly Ala Lys Cys Leu Pro Val Cys Cys Asn Arg Val Leu
305                 310                 315                 320

Gly Ile His His Ile Ser Val Met His Ser Ile Phe Ser Leu Leu Gly
                325                 330                 335

Leu Leu Gly Glu Ile Thr Tyr Ile Val Leu Leu Val Leu His Thr Val
            340                 345                 350

Trp Asn Gly Asn Gly Met Ile Gly Phe Gln Val Leu Leu Ser Ile Gly
        355                 360                 365

Glu Leu Ser Leu Ala Ile Val Ile Ala Leu Thr Ser Gly Leu Leu Thr
    370                 375                 380

Gly Leu Leu Leu Asn Leu Lys Ile Trp Lys Ala Pro His Val Ala Lys
385                 390                 395                 400

Tyr Phe Asp Asp Gln Val Phe Trp Lys Phe Pro His Leu Ala Val Gly
                405                 410                 415

Phe
```

<210> 149
<211> 417
<212> PRT
<213> RhCe

<220>
<223> Residue 121-135

<400> 149

```
Met Ser Ser Lys Tyr Pro Arg Ser Val Arg Arg Cys Leu Pro Leu Cys
 1               5                  10                  15

Ala Leu Thr Leu Glu Ala Ala Leu Ile Leu Leu Phe Tyr Phe Phe Thr
            20                  25                  30

His Tyr Asp Ala Ser Leu Glu Asp Gln Lys Gly Leu Val Ala Ser Tyr
        35                  40                  45

Gln Val Gly Gln Asp Leu Thr Val Met Ala Ala Ile Gly Leu Gly Phe
    50                  55                  60

Leu Thr Ser Ser Phe Arg Arg His Ser Trp Ser Ser Val Ala Phe Asn
65                  70                  75                  80

Leu Phe Met Leu Ala Leu Gly Val Gln Trp Ala Ile Leu Leu Asp Gly
                85                  90                  95

Phe Leu Ser Gln Phe Pro Ser Gly Lys Val Val Ile Thr Leu Phe Ser
            100                 105                 110

Ile Arg Leu Ala Thr Met Ser Ala Met Ser Val Leu Ile Ser Ala Gly
        115                 120                 125

Ala Val Leu Gly Lys Val Asn Leu Ala Gln Leu Val Val Met Val Leu
    130                 135                 140

Val Glu Val Thr Ala Leu Gly Thr Leu Arg Met Val Ile Ser Asn Ile
145                 150                 155                 160

Phe Asn Thr Asp Tyr His Met Asn Leu Arg His Phe Tyr Val Phe Ala
                165                 170                 175

Ala Tyr Phe Gly Leu Thr Val Ala Trp Cys Leu Pro Lys Pro Leu Pro
            180                 185                 190

Lys Gly Thr Glu Asp Asn Asp Gln Arg Ala Thr Ile Pro Ser Leu Ser
        195                 200                 205

Ala Met Leu Gly Ala Leu Phe Leu Trp Met Phe Trp Pro Ser Val Asn
    210                 215                 220

Ser Ala Leu Leu Arg Ser Pro Ile Gln Arg Lys Asn Ala Met Phe Asn
225                 230                 235                 240

Thr Tyr Tyr Ala Leu Ala Val Ser Val Val Thr Ala Ile Ser Gly Ser
                245                 250                 255
```

```
Ser Leu Ala His Pro Gln Arg Lys Ile Ser Met Thr Tyr Val His Ser
            260                 265                 270

Ala Val Leu Ala Gly Gly Val Ala Val Gly Thr Ser Cys His Leu Ile
        275                 280                 285

Pro Ser Pro Trp Leu Ala Met Val Leu Gly Leu Val Ala Gly Leu Ile
    290                 295                 300

Ser Ile Gly Gly Ala Lys Cys Leu Pro Val Cys Cys Asn Arg Val Leu
305                 310                 315                 320

Gly Ile His His Ile Ser Val Met His Ser Ile Phe Ser Leu Leu Gly
                325                 330                 335

Leu Leu Gly Glu Ile Thr Tyr Ile Val Leu Leu Val Leu His Thr Val
            340                 345                 350

Trp Asn Gly Asn Gly Met Ile Gly Phe Gln Val Leu Leu Ser Ile Gly
        355                 360                 365

Glu Leu Ser Leu Ala Ile Val Ile Ala Leu Thr Ser Gly Leu Leu Thr
    370                 375                 380

Gly Leu Leu Leu Asn Leu Lys Ile Trp Lys Ala Pro His Val Ala Lys
385                 390                 395                 400

Tyr Phe Asp Asp Gln Val Phe Trp Lys Phe Pro His Leu Ala Val Gly
                405                 410                 415

Phe
```

<210> 150
<211> 417
<212> PRT
<213> RhcE

<220>
<223> Residue 131-145

<400> 150

```
Met Ser Ser Lys Tyr Pro Arg Ser Val Arg Arg Cys Leu Pro Leu Trp
  1               5                  10                  15

Ala Leu Thr Leu Glu Ala Ala Leu Ile Leu Leu Phe Tyr Phe Phe Thr
```

20 25 30

His Tyr Asp Ala Ser Leu Glu Asp Gln Lys Gly Leu Val Ala Ser Tyr
35 40 45

Gln Val Gly Gln Asp Leu Thr Val Met Ala Ala Leu Gly Leu Gly Phe
50 55 60

Leu Thr Ser Asn Phe Arg Arg His Ser Trp Ser Ser Val Ala Phe Asn
65 70 75 80

Leu Phe Met Leu Ala Leu Gly Val Gln Trp Ala Ile Leu Leu Asp Gly
85 90 95

Phe Leu Ser Gln Phe Pro Pro Gly Lys Val Val Ile Thr Leu Phe Ser
100 105 110

Ile Arg Leu Ala Thr Met Ser Ala Met Ser Val Leu Ile Ser Ala Gly
115 120 125

Ala Val Leu Gly Lys Val Asn Leu Ala Gln Leu Val Val Met Val Leu
130 135 140

Val Glu Val Thr Ala Leu Gly Thr Leu Arg Met Val Ile Ser Asn Ile
145 150 155 160

Phe Asn Thr Asp Tyr His Met Asn Leu Arg His Phe Tyr Val Phe Ala
165 170 175

Ala Tyr Phe Gly Leu Thr Val Ala Trp Cys Leu Pro Lys Pro Leu Pro
180 185 190

Lys Gly Thr Glu Asp Asn Asp Gln Arg Ala Thr Ile Pro Ser Leu Ser
195 200 205

Ala Met Leu Gly Ala Leu Phe Leu Trp Met Phe Trp Pro Ser Val Asn
210 215 220

Ser Pro Leu Leu Arg Ser Pro Ile Gln Arg Lys Asn Ala Met Phe Asn
225 230 235 240

Thr Tyr Tyr Ala Leu Ala Val Ser Val Val Thr Ala Ile Ser Gly Ser
245 250 255

Ser Leu Ala His Pro Gln Arg Lys Ile Ser Met Thr Tyr Val His Ser
260 265 270

Ala Val Leu Ala Gly Gly Val Ala Val Gly Thr Ser Cys His Leu Ile

275 280 285

Pro Ser Pro Trp Leu Ala Met Val Leu Gly Leu Val Ala Gly Leu Ile
290 295 300

Ser Ile Gly Gly Ala Lys Cys Leu Pro Val Cys Cys Asn Arg Val Leu
305 310 315 320

Gly Ile His His Ile Ser Val Met His Ser Ile Phe Ser Leu Leu Gly
325 330 335

Leu Leu Gly Glu Ile Thr Tyr Ile Val Leu Leu Val Leu His Thr Val
340 345 350

Trp Asn Gly Asn Gly Met Ile Gly Phe Gln Val Leu Leu Ser Ile Gly
355 360 365

Glu Leu Ser Leu Ala Ile Val Ile Ala Leu Thr Ser Gly Leu Leu Thr
370 375 380

Gly Leu Leu Leu Asn Leu Lys Ile Trp Lys Ala Pro His Val Ala Lys
385 390 395 400

Tyr Phe Asp Asp Gln Val Phe Trp Lys Phe Pro His Leu Ala Val Gly
405 410 415

Phe

<210> 151
<211> 417
<212> PRT
<213> RhD

<220>
<223> Residue 141-155

<400> 151
Met Ser Ser Lys Tyr Pro Arg Ser Val Arg Arg Cys Leu Pro Leu Trp
1 5 10 15

Ala Leu Thr Leu Glu Ala Ala Leu Ile Leu Leu Phe Tyr Phe Phe Thr
20 25 30

His Tyr Asp Ala Ser Leu Glu Asp Gln Lys Gly Leu Val Ala Ser Tyr
35 40 45

```
Gln Val Gly Gln Asp Leu Thr Val Met Ala Ala Ile Gly Leu Gly Phe
    50                  55                  60

Leu Thr Ser Ser Phe Arg Arg His Ser Trp Ser Ser Val Ala Phe Asn
65                  70                  75                  80

Leu Phe Met Leu Ala Leu Gly Val Gln Trp Ala Ile Leu Leu Asp Gly
                85                  90                  95

Phe Leu Ser Gln Phe Pro Ser Gly Lys Val Val Ile Thr Leu Phe Ser
            100                 105                 110

Ile Arg Leu Ala Thr Met Ser Ala Leu Ser Val Leu Ile Ser Val Asp
        115                 120                 125

Ala Val Leu Gly Lys Val Asn Leu Ala Gln Leu Val Val Met Val Leu
    130                 135                 140

Val Glu Val Thr Ala Leu Gly Asn Leu Arg Met Val Ile Ser Asn Ile
145                 150                 155                 160

Phe Asn Thr Asp Tyr His Met Asn Met Met His Ile Tyr Val Phe Ala
                165                 170                 175

Ala Tyr Phe Gly Leu Ser Val Ala Trp Cys Leu Pro Lys Pro Leu Pro
            180                 185                 190

Glu Gly Thr Glu Asp Asn Asp Gln Thr Ala Thr Ile Pro Ser Leu Ser
        195                 200                 205

Ala Met Leu Gly Ala Leu Phe Leu Trp Ile Phe Trp Pro Ser Phe Asn
    210                 215                 220

Ser Ala Leu Leu Arg Ser Pro Ile Glu Arg Lys Asn Ala Val Phe Asn
225                 230                 235                 240

Thr Tyr Tyr Ala Val Ala Val Ser Val Val Thr Ala Ile Ser Gly Ser
                245                 250                 255

Ser Leu Ala His Pro Gln Gly Lys Ile Ser Lys Thr Tyr Val His Ser
            260                 265                 270

Ala Val Leu Ala Gly Gly Val Ala Val Gly Thr Ser Cys His Leu Ile
        275                 280                 285

Pro Ser Pro Trp Leu Ala Met Val Leu Gly Leu Val Ala Gly Leu Ile
    290                 295                 300
```

```
Ser Val Gly Gly Ala Lys Tyr Leu Pro Gly Cys Cys Asn Arg Val Leu
305                 310                 315                 320

Gly Ile Pro His Ser Ser Ile Met Gly Tyr Asn Phe Ser Leu Leu Gly
                325                 330                 335

Leu Leu Gly Glu Ile Ile Tyr Ile Val Leu Leu Val Leu Asp Thr Val
            340                 345                 350

Gly Ala Gly Asn Gly Met Ile Gly Phe Gln Val Leu Leu Ser Ile Gly
        355                 360                 365

Glu Leu Ser Leu Ala Ile Val Ile Ala Leu Thr Ser Gly Leu Leu Thr
    370                 375                 380

Gly Leu Leu Leu Asn Leu Lys Ile Trp Lys Ala Pro His Glu Ala Lys
385                 390                 395                 400

Tyr Phe Asp Asp Gln Val Phe Trp Lys Phe Pro His Leu Ala Val Gly
                405                 410                 415

Phe
```


<210> 152
<211> 417
<212> PRT
<213> Rhce

<220>
<223> Residue 151-165

<400> 152

```
Met Ser Ser Lys Tyr Pro Arg Ser Val Arg Arg Cys Leu Pro Leu Trp
  1               5                  10                  15

Ala Leu Thr Leu Glu Ala Ala Leu Ile Leu Leu Phe Tyr Phe Phe Thr
             20                  25                  30

His Tyr Asp Ala Ser Leu Glu Asp Gln Lys Gly Leu Val Ala Ser Tyr
         35                  40                  45

Gln Val Gly Gln Asp Leu Thr Val Met Ala Ala Leu Gly Leu Gly Phe
     50                  55                  60
```

```
Leu Thr Ser Asn Phe Arg Arg His Ser Trp Ser Ser Val Ala Phe Asn
 65                  70                  75                  80

Leu Phe Met Leu Ala Leu Gly Val Gln Trp Ala Ile Leu Leu Asp Gly
                 85                  90                  95

Phe Leu Ser Gln Phe Pro Pro Gly Lys Val Val Ile Thr Leu Phe Ser
            100                 105                 110

Ile Arg Leu Ala Thr Met Ser Ala Met Ser Val Leu Ile Ser Ala Gly
        115                 120                 125

Ala Val Leu Gly Lys Val Asn Leu Ala Gln Leu Val Val Met Val Leu
    130                 135                 140

Val Glu Val Thr Ala Leu Gly Thr Leu Arg Met Val Ile Ser Asn Ile
145                 150                 155                 160

Phe Asn Thr Asp Tyr His Met Asn Leu Arg His Phe Tyr Val Phe Ala
                165                 170                 175

Ala Tyr Phe Gly Leu Thr Val Ala Trp Cys Leu Pro Lys Pro Leu Pro
            180                 185                 190

Lys Gly Thr Glu Asp Asn Asp Gln Arg Ala Thr Ile Pro Ser Leu Ser
        195                 200                 205

Ala Met Leu Gly Ala Leu Phe Leu Trp Met Phe Trp Pro Ser Val Asn
    210                 215                 220

Ser Ala Leu Leu Arg Ser Pro Ile Gln Arg Lys Asn Ala Met Phe Asn
225                 230                 235                 240

Thr Tyr Tyr Ala Leu Ala Val Ser Val Val Thr Ala Ile Ser Gly Ser
                245                 250                 255

Ser Leu Ala His Pro Gln Arg Lys Ile Ser Met Thr Tyr Val His Ser
            260                 265                 270

Ala Val Leu Ala Gly Gly Val Ala Val Gly Thr Ser Cys His Leu Ile
        275                 280                 285

Pro Ser Pro Trp Leu Ala Met Val Leu Gly Leu Val Ala Gly Leu Ile
    290                 295                 300

Ser Ile Gly Gly Ala Lys Cys Leu Pro Val Cys Cys Asn Arg Val Leu
305                 310                 315                 320
```

```
Gly Ile His His Ile Ser Val Met His Ser Ile Phe Ser Leu Leu Gly
                325                 330                 335

Leu Leu Gly Glu Ile Thr Tyr Ile Val Leu Leu Val Leu His Thr Val
            340                 345                 350

Trp Asn Gly Asn Gly Met Ile Gly Phe Gln Val Leu Leu Ser Ile Gly
        355                 360                 365

Glu Leu Ser Leu Ala Ile Val Ile Ala Leu Thr Ser Gly Leu Leu Thr
    370                 375                 380

Gly Leu Leu Leu Asn Leu Lys Ile Trp Lys Ala Pro His Val Ala Lys
385                 390                 395                 400

Tyr Phe Asp Asp Gln Val Phe Trp Lys Phe Pro His Leu Ala Val Gly
                405                 410                 415

Phe
```